ONCOLOGY

Expression of VEGF and VEGFR2 in Tumors during Neoadjuvant Therapy of Patients with Breast Cancer

E. A. Kim, E. S. Gershtein, I. V. Vysotskaya, and N. E. Kushlinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 2, pp. 206-209, February, 2008
Original article submitted July 4, 2007.

We revealed a significant positive correlation between the concentrations of tissue VEGF and its receptor VEGFR2 in the primary tumor. The degree and direction of changes in the content of VEGF and VEGFR2 in cytosols of tumors from patients with locally spread breast cancer during the preoperation therapy depended on initial levels of these parameters. We found that the expression of the studied factors depended on the degree of therapeutic pathomorphosis in the tumor tissue.

Key Words: *VEGF, VEGFR2, angiogenesis; breast tumor; neoadjuvant therapy*

The formation of new blood vessels (neoangiogenesis) is necessary for tumor grown and invasion [9] and plays an important role in the development of metastases [8]. The study of inductors and blockers of neovascularization in tumor growth is an important aspect of experimental and clinical oncology. Factors modulating tumor progression can have a prognostic value, which allows to divide patients into groups with different risk and to choose adequate therapy for them [1,3,5]. Moreover, study of the relationships between the level of angiogenic factors and tumor growth and metastasizing provides the possibility of the search, development, and introduction of prospective targeted drugs [1,5].

VEGF (vascular endothelial growth factor), a specific mitogen for endothelial cells, acts as a regulator of vascular permeability, and induces expression of antiapoptotic proteins (Akt and Bcl-2) [1,5,7], which is an important event in initiation of neoangiogenesis. Few antiogenic factors are expressed in breast cancer (BC), but the concentration of VEGF in the tumor far surpasses the concentrations of other factors [12]. VEGF is more frequently and more intensively expressed in the tumor tissue than in unchanged adjacent tissue, which attests to its prognostic value in BC [2,4]. VEGF realizes its effect via specific tyrosine kinase receptors VEGFR1 (flt-1) and VEGFR2 (KDR/flk-1) [4,6]. PCR analysis showed that VEGFR2 predominates in tumor cells; it interacts with its ligand VEGF and via a paracrine mechanism transmits the signal stimulating proliferation [13].

Some independent study groups showed that high VEGF content in the tumor correlates with low total and relapse-free survival in various clinical groups. Unfavorable prognostic role of VEGF was most often reported in patients with early stages of the disease without metastases into lymph nodes [10,11]. The expression of VEGF and its receptors in the tumor tissue in patients with locally spread BC is little studied; the majority of reports present only statistic data, *i.e.* markers of angiogenesis were measured only once, primarily before the start of specific treatment.

The aim of the present study was to evaluate the effect of preoperation therapy on the content of VRGF and VRGFR2 in tumors of patients with locally spread BC and to compare the dynamics of these parameters with the effect of treatment.

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** helene-kim@yandex.ru. E. A. Kim

MATERIALS AND METHODS

The study comprised 30 patients aged 24-69 years (mean age 49 years) with primary BC T2-4N0-3M0 stages (according to TNM classification) observed and treated at N. N. Blokhin Cancer Research Center in 2005-2006. Complex examination was performed before the start of specific treatment in all cases. In included measurement of the content of VEGF and VEGFR2 in the cytosol from biopsy samples of the tumor. The patients were divided into 3 groups depending on the neoadjuvant therapy: 10 patients of group 1 received 2 courses of preoperation polychemotherapy (FAC protocol); in group 2 patients (n=10), neoadjuvant chemoradiotherapy (FAC+radiotherapy) was performed with a single focal dose (SFD) of 3 Gy and total focal dose (TFD) of 20-40 Gy (isoeffective doses); 10 patients comprising group 3 received radiotherapy alone on the mammary gland and zones of regional metastasizing (SFD 3 Gy×10-11 fractions to TFD 28-40 Gy (isoeffective doses). After 2.5-3.0 weeks, radical surgery (mastectomy of resection depending on the attained clinical and X-ray effect) was performed to all patients.

The dynamics of expression of VEGF and VEGFR2 in the tumor was evaluated by analyzing biopsy samples before and after surgery. The samples were treated as described previously [6]. The content of VEGF and VEGFR2 in cytosols of tumors was measured using Human VEGF ELISA (BioSource International) and Quantikine® Human VEGFR2 Immunoassay (R&D systems) kits. The concentration of factors was expressed in pg/mg cytosol protein (protein concentration was measured by the method of Lowry).

General clinical and morphological prognostic criteria were also evaluated: histological type of the tumor, degree of therapeutic pathomorphosis, number of involved lymph nodes, receptor status of the tumor (estrogen and progesterone receptors) before and after specific treatment, expression of HER-2/neu.

Comparison and analysis of the relationships were performed using nonparametric Wald—Wolfowitz and Mann—Whitney tests, median test, and Spearman rank correlation test. The data were processed using Statistica 6.0 software (StatSoft Inc).

RESULTS

Both factors were detected in all tumor samples. The contents of VEGF and VEGFR2 before treatment were 10.7-858 and 8.4-606 pg/mg, respectively (corresponding medians were 107 and 165 pg/mg). A direct correlation was found between the level of VEGF and its receptor in the tumor tissue (Fig. 1; R=0.39; p<0.05).

After preoperation therapy, the level of VEGF in the tumor decreased by 7-89% (p<0.05) in 16% patients. In other patients, the content of VEGF increased, but these changes were insignificant. Changes in VEGF content in the tumor depended on menstrual status of the patient: in postmenopausal patients (n=13, 43.3%) the content of VEGF usually decreased after treatment (median 55.3 pg/mg protein), while in 10 young menstruating patients (33.3%) the content of cytosolic VEGF increased (median 26.6 pg/mg protein, p=0.01).

We revealed no correlations between the direction and degree of observed changes on the one hand and clinical stage of the process, malignancy, histological type of the tumor, and HER-2/neu status, on the other.

A clear-cut relationship was found between the concentrations of VEGF in the tumor after treatment and the degree of therapeutic pathomorphosis determined during histological examination: more pronounced changes in the tumor (grade IV of therapeutic pathomorphosis) corresponded to lower content of VEGF in the residual tumor (Table 1; p<0.05).

Analysis of VEGF content before and after specific treatment revealed the following relationship: the content of VEGF after treatment tended to decrease in patients with VEGF concentration in the primary tumor >120 pg/mg protein and little changes in patients with initially low VEGF content (<120 pg/mg protein). VEGFR2 underwent similar changes during neoadjuvant therapy: it significantly increased after therapy in 17 patients with initially

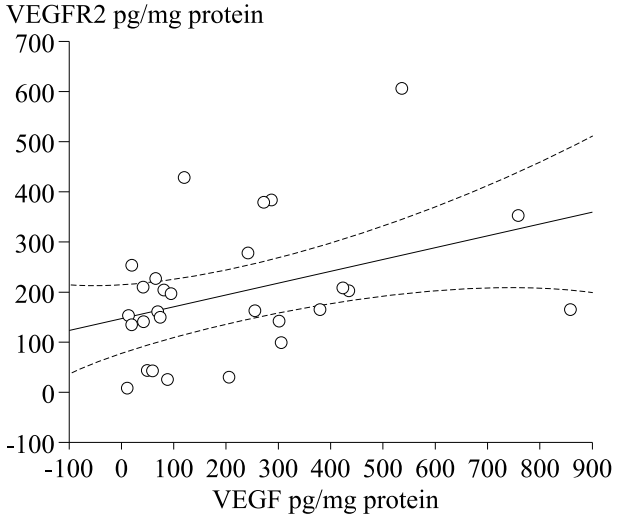

Fig. 1. Correlation between the content of VEGF and VEGFR2 in cytosols of tumors in patients with BC before treatment (R=0.39; p<0.05 Spearman rank test).

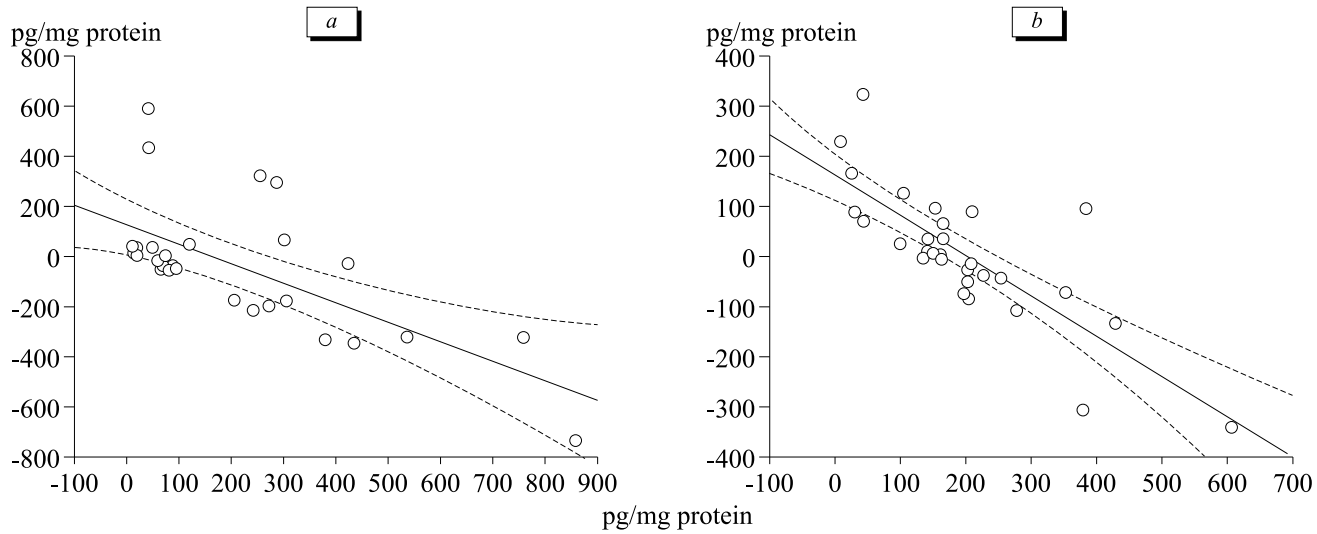

Fig. 2. Changes in the contents of VEGF (*a*) and VEGFR2 (*b*) in the tumor depending on their initial levels (R=-0.67; R=-0.79, respectively, p<0.05 Spearman rank test).

low level of this protein in the primary tumor (<200 pg/mg protein) and significantly deceased in others.

In 14 patients, the concentration of VEGFR2 after neoadjuvant therapy decreased by 2-81%, in other cases this parameter increased. The content of VEGFR2 after treatment also depended on the menstrual status of patients: the concentration of VEGFR2 in the tumor remained high in patients with preserved menstrual functions (152-479 pg/mg protein, median 202 pg/mg protein) compared to

TABLE 1. Content of Free VEGF in Cytosols of Tumors with Different Degree of Therapeutic Pathomorphosis after Specific Treatment

Degree of pathomorphosis	Number of patients	VEGF, pg/mg protein		Median, quartiles
		range	*M*±*m*	
0-I	6	25.6-582.1	297.6±238.5	367.2 77.3±436
II	10	14.0-577.6	211.9±211.6	123.3 75.0-394.9
III	9	27.4-632.2	145.1±194.1	51.5 42.6-168.9
IV	5	24.1-54.5	40.6±14.0	46.5 21.7-51.0

Note. p<0.05 median test.

TABLE 2. Content of VEGFR2 in Cytosols of Tumors after Specific Treatment Depending on the Number of Involved Lymph Nodes

Involved nodes (N)	Number of patients	VEGFR2, pg/mg protein		Median, quartiles
		range	*M*±*m*	
N0	4	118.9-157.3	138.0±21.7	137.9 119.4-156.5
N1	11	114.1-479.4	221.6±108.5	176.8 158.6-234.4
N2	12	72.8-299.3	194.5±73.5	194.7 129.9-243.5
N3	3	191.7-265.5	235.5±38.8	249.4 220.5-257.4

Note. p=0.03 for comparison of groups 1 and 4, Mann—Whitney test.

premenopausal patients (72.8-249 pg/mg protein, median 132 pg/mg; $p<0.05$)

Further analysis of the content of neoangiogenesis factors after therapy found no significant relationships with such prognostically relevant parameters as stage of the tumor process and size of primary tumor, but revealed a dependence of VEGFR2 expression after treatment on the number of involved lymph nodes (Table 2). In patients with multiple metastases into regional lymph nodes (according to data of histological examination of the operation material), the content of VEGFR2 in the primary tumor was significantly higher than in patients with BC without metastases into lymph nodes ($p=0.03$).

It should be noted that VEGF expression and the content of its receptor in the tumor correlated with the degree of therapeutic pathomorphosis: the concentration of VEGFR2 in tumors with grade IV pathomorphosis was lower than in tumors with grade 0-I pathomorphosis ($p=0.04$).

The decrease in VEGF and VEGFR2 content in the tumor significantly correlated with their initial levels: the higher was the initial concentration of VEGF and VEGFR2 in the tumor, the more pronounced decrease was noted after neoadjuvant therapy (Fig. 2).

The effect of the receptor status of the tumor on changes in the expression of the studied angiogenic factors during specific treatment is disputable. In the group of patients with tumors negative by both estrogen and progesterone receptors, the level of VEGFR2 more often decreased after treatment than in patients with receptor-positive tumors ($p<0.05$). Similar tendency was found for the content of free VEGF in the tumor, but the difference was insignificant.

Comparison of changes in VEGF content in tumor samples from patients receiving different preoperation treatment showed that this parameter increased by 22-1032% (median 48%) in 70% patients receiving chemotherapy and decreased by 7-89% (median 59%) in 80% patients receiving radiochemotherapy. After neoadjuvant therapy, the content of VEGF in the tumor decreased only in 50% cases (by 28-78%, median 53%). Similar data were obtained for changes in the level of VEGFR2 in the tumor tissue in the corresponding groups, but the differences were insignificant.

On the whole, the direction and degree of changes in the concentrations of VEGF and VEGFR2 in tumors did not depend on the type of preoperation treatment.

REFERENCES

1. E. C. Gershtein and N. E. Kushlinskii, *Mammology*, No. 1. 65-70 (2005).
2. E. C. Gershtein, A. M. Shcherbakov, S. K. Alieva, *et al., Byull. Eksp. Biol. Med*., **135**, No. 1, 99-102 (2003).
3. E. C. Gershtein, A. M. Shcherbakov, S. K. Alieva, *et al., Vestn. Ross, Onkol. Nauch. Center im. N. N. Blokhin RAMS,* Nos. 1-2. 26-30 (2005).
4. L. G. Zhukova and M. R. Lichinitser, *Byull. Eksp. Biol. Med*., **135**, No. 5, 562-565 (2003).
5. N. E. Kushlinskii and E. C. Gershtein , *Ibid*., **133**, No. 6, 521-528 (2002).
6. A. M. Shcherbakov, E. C. Gershtein, O. A. Anurova, and N. E. Kushlinskii, *Vopr. Onkol*., **51**, No. 3, 317-321 (2005).
7. N. Ferrara, *Breast Cancer Res. Treat.*, **36**, No. 2, 127-137 (1995).
8. I. J. Fidler and L. M. Ellis, *Cell*, **79**, No. 2, 185-188 (1994).
9. J. Folkman, *Nat. Med.*, **1**, No. 1, 27-31 (1995).
10. G. Gasparini, *Oncologist*, **5**, Suppl. 1, 37-44 (2000).
11. B. Linderholm, B. Tavelin, K. Grankvist, R. Heriksson, *J. Clin. Oncol.*, **16**, No. 9, 3121-3128 (1998).
12. M. Relf, S. LeJeune, P. A. Scott, *et al*., *Cancer Res*., **57**, No. 5, 963-969 (1997).
13. V. Speirs and S. L. Atkin, *Br. J. Cancer*, **80**, Nos. 5-6, 898-903 (1999).